size but the ducts and intromittent organ were rudimentary. The accessory glands were represented by small ducts without glandular portion. Secondary spermatocysts became evident in the follicles but they were lesser in number and were crowded among the spermatogonia.

Dhondt *et al*[2] reported adultoids in rhinoceros beetles as a result of JH analogue treatment. The present study reveals that the differentiation of male reproductive system in adultoids of *O. rhinoceros* is inhibited as a result of methoprene treatment. Methoprene applied to early pupae of *O. rhinoceros* showed a dose-dependent inhibition of male reproductive system and sperm differentiation. The inhibitory action of JH analogues on the male reproductive system has been reported in *Trogoderma granarium*[3], *Spodoptera littoralis*[4], and *Scirpophaga incertulas*[5]. When the dose applied was reduced in *O. rhinoceros* a reduced number of differentiating spermatocytes were seen as reported in *Corcyra cephalonica*[6]. In *O. rhinoceros* methoprene also inhibited the development and differentiation of male accessory glands.

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EFFECT OF PENICILLIC ACID ON ISOLATED FROG HEART

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PENICILLIC acid, an α,β-unsaturated conjugated lactone is produced by various food-borne fungi[1]. Earlier investigations revealed that penicillic acid is carcinogenic in rats and mice[2], cytotoxic in cultured cells[3] and hepatotoxic in mice[4]. Penicillic acid effects on erythrocytes[5] and on intestinal brush border of rabbits[6] have been reported recently. Murnaghan[7] reported that penicillic acid has a digitalis-like effect on cardiac muscle. Digitalis increases the force of contraction (positive ionotropic action) but slows the ventricular rate[8]. Based on this, the present work was undertaken to investigate the effect of penicillic acid on heart tissue using isolated frog's heart as the experimental model.

Isolation and purification of penicillic acid have been reported earlier[6]. Frogs (*Rana hexadactyla*) with a live weight of 100–120 g were used for isolation and perfusion of heart.

Heart was perfused with Ringer's solution through a cannula tied into the inferior vena cava, the cannula was connected to perfusion funnel and the funnel was opened into the cannula. The height of the funnel and the cannula was adjusted so that the fluid remained in the vertical limb of the cannula to about three-fourths of its height. The apex of the heart was hooked to a recording lever. The rhythmic beating of the heart was recorded using a smoked drum of a kymograph[9].

To study the effects of penicillic acid at various levels, different concentrations (1 mg/ml, 1.5 mg/ml, and 2 mg/ml) were prepared in Ringer's solution.

Isolated heart was perfused, first with Ringer's solution and the normal recording done[9]. This was followed by perfusion with various concentrations of penicillic acid as mentioned above. The effect of the perfusate was recorded systematically on the kymograph. In the present study, six isolated frogs' hearts were used and recordings from the six frogs showed consistent results. The figure shows one such recording.

Murnaghan[7] reported that penicillic acid in concentrations of 10^{-3} g/ml and 10^{-4} g/ml exerted a digitalis-like effect on cardiac tissue. The same author substantiated this by noting that penicillic

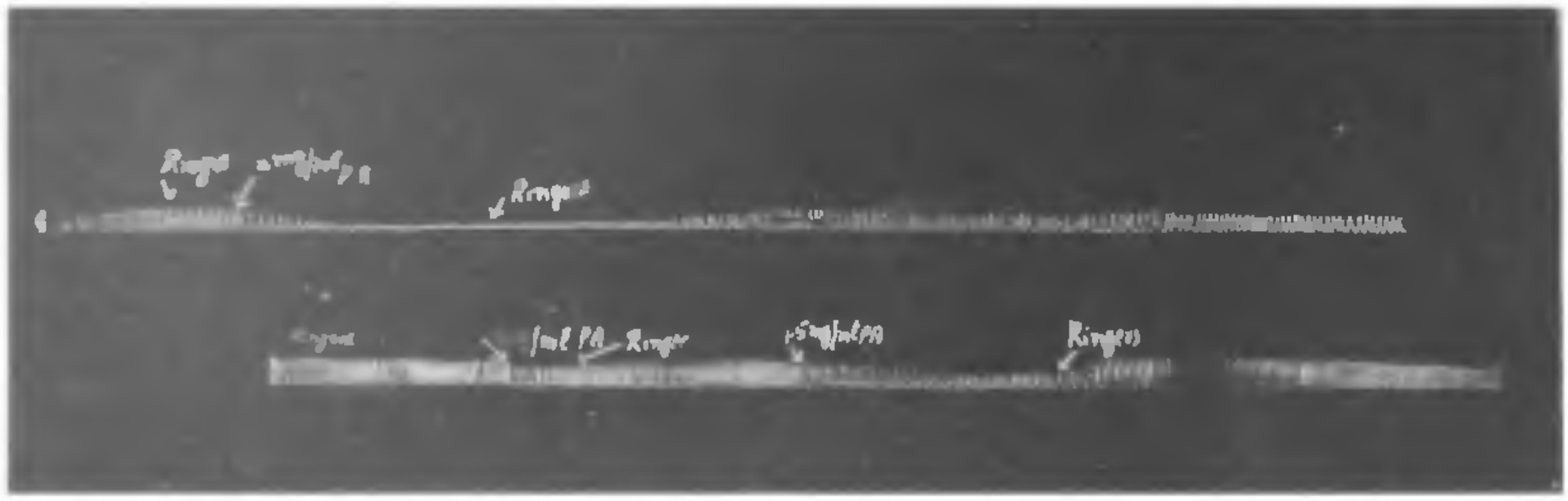

Figure 1. Kymograph recording of frog heart with Ringer's solution and penicillic acid.

acid increased the amplitude of the heartbeat without much effect upon the rate. After some time the systolic height of contractions began to decrease and simultaneously the diastolic relaxation became incomplete. The heart began to beat irregularly and finally the ventricle stopped in systolic contracture. Further in two other preparations of the same experiments using rabbit auricle and the cat heart perfusion through aorta, similar effects on amplitude and rate were noted and the systolic arrest was irreversible in all the three experiments.

In the present investigations, it was noted that penicillic acid at 1 mg/ml level retarded the heart rate and decreased the amplitude, at 1.5 mg/ml the effect was more pronounced and at 2 mg/ml level there was almost total cessation of heartbeat (figure 1). At all the three concentrations, it can be noted clearly from the recordings that the heart could be revived back to almost normal level when it was perfused with Ringer's solution. The new finding therefore is that at toxic levels there is almost a total cardiac arrest but subsequent perfusion with Ringer's solution washes out the toxic effects and heartbeat can be restored to its near normal condition.

Stoppage of heartbeat could possibly be due to the effect of penicillic acid on the Na^+, K^+-activated ATPase as reported by Phillips *et al*[10] in swine brain tissue. They also observed that repeated washings, partially restored the enzyme activity. From the present study also, it is clear that although penicillic acid has an inhibitory effect on heartbeat which may be due to the effect on Na^+, K^+-activated ATPase enzyme system essential for action potential, the same can be offset by repeated washing. Further it proves that Na^+, K^+-activated ATPase binding with penicillic acid is not irreversible.

Digitalis is well known to increase the force of contractions assessed by the amplitude when recorded but decreases the rate, but in the present study there is not only a decrease in the rate and reduction of amplitude but also an ultimate cardiac arrest at toxic levels. These effects only show that the digitalis-like effect on cardiac muscle may not be tenable *in toto*, as postulated by Murnaghan[7].

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